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Review

A review of COVID-19: Treatment strategies and CRISPR/Cas9 gene editing technology approaches to the coronavirus disease

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ARTICLE INFO

Article history:

Received 26 August 2021

Revised 12 September 2021

Accepted 5 October 2021

Available online xxx

Keywords:

CRISPR/Cas9

COVID-19

SARS-CoV-2

Treatment strategies

Pandemic

Public health

ABSTRACT

The new coronavirus SARS-CoV-2 pandemic has put the world on lockdown for the first time in decades. This has wreaked havoc on the global economy, put additional burden on local and global public health resources, and, most importantly, jeopardised human health. CRISPR stands for Clustered Regularly Interspaced Short Palindromic Repeats, and the CRISPR associated (Cas) protein (CRISPR/Cas) was identified to have structures in *E. coli*. The most modern of these systems is CRISPR/Cas. Editing the genomes of plants and animals took several years and cost hundreds of thousands of dollars until the CRISPR approach was discovered in 2012. As a result, CRISPR/Cas has piqued the scientific community's attention, particularly for disease diagnosis and treatment, because it is faster, less expensive, and more precise than previous genome editing technologies. Data from gene mutations in specific patients gathered using CRISPR/Cas can aid in the identification of the best treatment strategy for each patient, as well as other research domains such as coronavirus replication in cell culture, such as SARS-CoV2. The implications of the most prevalent driver mutations, on the other hand, are often unknown, making treatment interpretation difficult. For detecting a wide range of target genes, the CRISPR/Cas categories provide highly sensitive and selective tools. Genome-wide association studies are a relatively new strategy to discovering genes involved in human disease when it comes to the next steps in genomic research. Furthermore, CRISPR/Cas provides a method for modifying non-coding portions of the genome, which will help advance whole genome libraries by speeding up the analysis of these poorly defined parts of the genome.

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Peer review under responsibility of King Saud University.



Production and hosting by Elsevier

<https://doi.org/10.1016/j.sjbs.2021.10.020>

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Please cite this article as: Muhammad Farhat Ullah, Y. Ali, Muhammad Ramzan Khan et al., A review of COVID-19: Treatment strategies and CRISPR/Cas9 gene editing technology approaches to the coronavirus disease, Saudi Journal of Biological Sciences, <https://doi.org/10.1016/j.sjbs.2021.10.020>

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1. Background information

COVID-19 was declared a pandemic by the World Health Organization on March 11, 2020. The World Health Organization has declared COVID-19 a Public Health Emergency of International Concern (Qu et al., 2019). As of today, July 8, 2020, the COVID-19 outbreak has spread to 215 nations, according to the World Health Organization. COVID-19 infection has been linked to 539,026 deaths worldwide, with 11,635,939 verified cases. The US had the most deaths (129,963), followed by Brazil (65,487), Italy (34,869), Mexico (31,119), Spain (28,388), India (20,642), Iran (11,931), and China (11,931). (11,931). (65,487). (11,931). (4648). A total of 2,923,432 persons have been infected in the United States, making it the pandemic's most impacted country (Mehravar et al., 2018). These figures are constantly increasing. Unsurprisingly, the rapid increase in the number of infected people has sparked significant fear among the general public. Since the outbreak began, scientists all around the world have been researching for possible detection and treatment options. Prof. Yong-Zhen Zhang and colleagues published the first genome of COVID-19 on January 10th in this regard. The year 2020 (Hidalgo-Cantabrana et al., 2018). CRISPR is an adaptive immuno-

logical response in bacteria and archaea that uses endonuclease to accurately target and cut down viral DNA. Scientists could make very precise genetic modifications based on the type of cell by reengineering this immune response to target certain portions of genetic information. CRISPR therapeutic and diagnostic platforms are built on this foundation (Pan and Kraschel, 2018). In archaea and bacteria, CRISPR and CRISPR-associated proteins guard against phages, plasmids, and other foreign DNA elements with invading nucleic acids, DNA, or RNA. So far, CRISPR-Cas systems have been divided into two categories, six types, and 21 subtypes (Hidalgo-Cantabrana et al., 2018). The repetition sequences are between 25 and 40 nucleotides long, while the spacer sequences are between 21 and 71 nucleotides long. (Qu et al., 2019). To ensure its efficacy, several approaches for targeted gene editing in cell and animal models have been devised. Two examples are Zinc-Finger Nucleases (ZFNs) and Transcription Activator-Like Effector Nucleases (TALENs). CRISPR/Cas (Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR/Cas) is a fast, simple, and successful gene editing technology (Mehravar et al., 2018). As a result, it's a powerful tool for modifying the genomes of a wide range of organisms that's also simple to use (Zhan et al., 2019). We can comprehend the benefit of genome editing technologies, which allow us to modify genomic DNA in a targeted manner utilising CRISPR/Cas systems, based on these scientific facts (Herai, 2019). The study also relies on the coexpression of a bacterial Cas nuclease and a guide RNA (gRNA) sequence. CRISPR/Cas gene editing, which is based on the coexpression of a bacterial Cas nuclease and a guide RNA, has allowed precise changes to be performed in a variety of genomes, including dsDNA viral genomes (gRNA) Because gRNA targeted Cas generates a DSB in the genome, two primary repair pathways compete to fix it, causing some sequence alterations (King and Munger, 2019). To reduce gene expression, researchers have relied on the creation of systemic and conditional gene knockouts in recent decades, particularly in coronaviruses (de Wilde et al., 2018). This review paper focus on the application of CRISPR/Cas techniques and their general immunological signalling, as well as gene editing technologies against emerging diseases such as coronavirus. As a result, we conducted a comprehensive review of potential COVID-19 detection and treatment

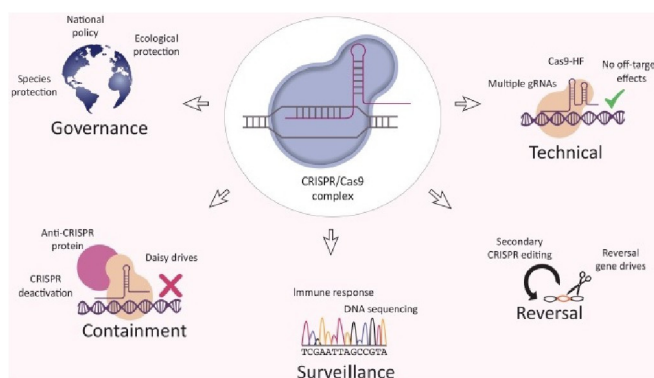


Fig. 1. Promoting genome editing safety (Pineda et al., 2018).

approaches. In the interest of public health, it could also serve as a good foundation for various government and private organisations to improve COVID-19 and other virus diagnosis, treatment, and prevention strategies. (See Fig. 1)

2. CRISPR/CAS and immune cells: Current status and significance

2.1. CRISPR/Cas systems types

CRISPR/Cas systems are divided into two categories: those that use multi-protein effector complexes and those that use single-protein effectors. Class 1 is made up of types I, III, and IV, whereas class 2 is made up of types II, V, and VI. It can also be divided into 19 different subtypes, with more CRISPR/Cas systems likely in the future. A type II single-protein effector derived from *Streptococcus pyogenes* is the most commonly utilised corporate Cas protein for functional broadcast (SpCas9). It's a type of guide RNA that helps the target gene get cleaved (Ford et al., 2019). Because type I and III CRISPR/Cas systems have several Cas proteins, whereas type II CRISPR/Cas systems only have the Cas9 protein, this result was reached (Makarova et al., 2011).

2.1.1. In human pluripotent stem cells, CRISPR was used

In the last few centuries, World has been hit hard by significant challenges like diseases and hunger. Humans continued to put efforts to get rid of the diseases and fulfill the needs of food security. Although many advancements have been made, with several milestones being achieved, the available technologies are still facing many difficulties in controlling diseases and better food security. In the past, many technologies provided rays of hope but soon vanished with a lot of loopholes. However, in recent times, the CRISPR technology implementation enhances the hopes of many solutions and challenges regarding disorders and scarcity of food (Ogaugwu et al., 2019). A different human cell has been cultured in the modified medium of Dulbecco Eagle such as immortalized MRC5-hTERT fibroblast with supplementation of 4.5 g/L glucose 1% penicillin-streptomycin, and fetal bovine serum (King and Munger, 2019).

2.1.2. CRISPR-Cas-mediated genome editing: A overview

TALEN, Case9, and ZFN, like engineered nucleases, actively participate in modifications by breaking double-stranded DNA in specific genomic loci, which is then repaired by one of at least two cellular DNA modifications pathways, namely homology-directed repair (HDR) and non-homologous end-joining (NHEJ). In the last two decades, the discovery of transcription activators

like effectors (TALEs) and programmable DNA binding proteins such as Zinc fingers (ZFs) resulted in the successful development of genome modifying equipment. Zfs are Cys2-His (C2H2) rich sequences, unlike DNA binding domains that are acquired from a transcription factor's family of Eukaryotes (Rahman et al., 2019). In prokaryotic cells, adaptive immune response exists in Cas9 proteins that inspired CRISPR genome-editing technology. CRISPR-Cas9 genome editing technology is more advanced and efficient compared to conventional genome-editing technologies. Besides the simplicity and efficiency in target designing and regulation, it can target multiple genes at the same time.

Small guided RNA (sgRNA) and Cas proteins are the two significant components of CRISPR. The sequence in sgRNA is also sometimes referred to as the scaffold. sgRNA contains target-specific spacer sequences and Cas enzyme attachment site. These sequences are concurrently crowded with 20 nucleotides, proto-spacer adjacent motif (PAM), Cas9 target sequence and a short DNA sequence of 2–6 base pairs, respectively. The Cas9 enzyme specifies the length of the PAM sequence (Kaushik et al., 2019).

2.1.3. Efficiency in editing

The efficiency of the editing techniques majorly drives the difficulties in genome editing. Advance genome editing technologies have minimized the errors with improvements in genome editing's therapeutic efficacy by improving the editing rate. The double-strand break restoration pathways in CRISPR-Cas systems act as regulatory components that significantly increase the editing rate. The editing efficiency among different cellular states and types vary due to the difference in endogenous cellular DNA repair mechanisms based on HDR and NHEJ. The mechanism of activation in end-joining (NHEJ) and homology-directed repair is different in the whole cell cycle, NHEJ being active in the whole-cell cycle, makes it more efficient in knocking out carcinogenic genes and generating indels, unlike the operation of HDR that varies in different cell cycle phases like S/G2. The HDR efficiency is DNA template type and homology arm length-dependent and more precisely, is relatively slow. HDR is based on the single-strand oligonucleotides or a plasmid containing alleles, and the transformation of the HDR template depends on the viral and non-viral vectors (Chen et al., 2019).

2.1.4. CRISPR Cas9 tools

Commercial and Academic institutes around the World have made an available variety of CRISPR resources and online tools. Table 1 reflects the number of widely utilized resources with its URL, including more detailed documentation of all the resources. These CRISPR resources play an unprecedented role in identifying and recognizing the suitable gRNA, listing detailed information

Table 1

The following is a list of commonly used CRISPR resources (Thomas et al., 2019).

S.No.	Name	URL
1	Addgene	http://www.addgene.org/crispr/
2	Benchling	https://benchling.com/crispr
3	BreakingCas (Khatodia et al., 2016)	http://bioinfogp.cnb.csic.es/tools/breakingcas/
4	Broad Institute GPP	https://portals.broadinstitute.org/gpp/public/
5	CHOPCHOP (Yamaguchi and de Lecea, 2018)	http://chopchop.cbu.uib.no/
6	CRISPOR (Ford et al., 2019)	http://crispor.net/
7	CrisFlash (Makarova et al., 2011)	https://github.com/crisflash/crisflash
8	Deskgen	https://www.deskgen.com
9	E-CRISP (Deveau et al., 2008)	http://www.e-crisp.org/E-CRISP/
10	Horizon Discovery	https://dharmacon.horizon-discovery.com/gene-editing/crispr-cas9/crispr-design-tool/
11	IDT	https://sg.idtdna.com/site/order/designtool/index/CRISPR_CUSTOM
12	Microsoft Research CRISPR (Ogaugwu et al., 2019)	https://crispr.ml
13	RGEN Tools (Herai, 2019)	http://www.rgenome.net/
14	Synthego	http://design.synthego.com
15	WTSI Genome Editing (WGE) (Zhan et al., 2019)	https://www.sanger.ac.uk/htgt/wge/

Table 2

Treatment Options for Anti-COVID-19 Repurposing in Development—Completed and Ongoing Clinical Trials.

Options for repurposing anti-COVID-19 drugs	In vitro anti-2019-nCoV activity was determined (+) or not (–)	The number of trial registrations	The current phase of the trial	In clinical trial/s, the following regimen was employed.	Reference
Lopinavir + ritonavir	+	ChiCTR2000029539	Completed (rejected)	100 mg ritonavir PO BD for 14 days	(Registry CCT, 2020b)
Favipiravir	+	ChiCTR2000030254	Completed (recommended for use in China and Japan)	400 mg lopinavir orally For the first day of the trial, 1600 mg/per dosage PO bid was administered, then 600 mg/dose PO bid until the trial ended..	(Registry CCT, 2020c)
Remdesivir	+	NCT04292899	Phase III	For one day, give 200 mg/dose OD IV, then give 100 mg/dose OD IV for the next 4–9 days.	(NIH, 2020r)
Arbidol	–	NCT04260594	Phase IV	Two tablets POTID for 10–14 days	(NIH, 2020e)
Chloroquine Phosphate	+	ChiCTR2000029542	–	500 mg/dose PO BID for not more than ten days	(Registry CCT, 2020d)
Hydroxychloroquine	+	ChiCTR2000029559	Completed	200 mg/dose TID PO for 5 days	(NIH, 2020f)
Carrimycin	–	NCT04261517	Phase IV	–	(NIH, 2020s)
Hydroxychloroquine-Azithromycin	–	NCT04286503	Completed	For ten days, take 200 mg of hydroxychloroquine PO TID.	(Register ECT, 2020)
		2020–000890-25, French National Agency for Drug Safety		Azithromycin: 500 mg PO on day one, then 250 mg every four days for the next four days.	
Tocilizumab	–	ChiCTR2000029765	Completed Phase II	4–8 mg/kg IV diluted in NS (single dose)	(NIH, 2020v, Registry CCT, 2020a)
Sarilumab	–	NCT04315480	Phase II	Single-dose IV	(NIH, 2020h)
Bevacizumab	–	NCT04315298	Phase II	500 mg in 100 ml NS IV rip	(NIH, 2020d)
Favipiravir + tocilizumab	–	NCT04275414	–	Favipiravir (favipiravir): 1600 mg/dose BD for 2 days, then 600 mg/dose BD for 7 days 4–8 mg/IV tocilizumab	(Registry CCT, 2020e)
INF- α + ribavirin, INF- α + ribavirin + LPR/RTR	–	ChiCTR2000029387	Not completed	INF- α : atomized inhalation, 5 million U/50 μ g per dose, BID for 14 days	(Registry CCT, 2020f)
INF- α 2 β	–	NCT04293887	Phase I	10 μ g BD in a nebulized solution	(NIH, 2020g)
RhACE2 (APN01)	–	NCT04335136	Phase II	–	(NIH, 2020)
NK cells	–	NCT04280224	Phase I	0.1–0.2 \times 10 ⁷ cells/kg of body weight twice weekly	(NIH, 2020k)
Thalidomide	–	NCT04273529	Phase II	100 mg PO every night for 14 days	(NIH, 2020t)
Fingolimod	–	NCT04280588	Phase II	0.5 mg PO OD for 3 consecutive days	(NIH, 2020i)
Dexamethasone*	–	NCT04395105	Phase III	16 mg IV for first 5 days followed by 8 mg IV for next 5 days	(NIH, 2020w)
Enoxaparin**	–	NCT04359277	Phase III	1 mg/kg every 12 h SC	(NIH, 2020a)

for the selection of gRNAs for experimental purposes. Some tools help mention gRNA for specific practical applications, while others count it as a part of the whole experimental design, starting with target location identification (Thomas et al., 2019). (See Tables 2, 3).

2.1.5. CRISPR application and design

CRISPR/Cas systems are primarily dependent on gRNA's ability to mark a particular target sequence. However, its applications are not implicitly reliant on the cleavage of Cas-endonucleases. The solicitation of the knock-out and knock-in is based on the formation of DSB that deletes or either inserts DNA (Thomas et al., 2019, Fu et al., 2019). The designing of CRISPR systems explicitly relies on basic principles like suitable target regions identification, selecting suitable gRNA based on on-target and off-target region and its scoring. These principles are primarily applicable for the large-scale designing approaches and targeted designing even though the selection of gRNAs can also be the meticulous difference amongst these two tactics (Thomas et al., 2019). Molecular characterization, response to a drug, screening of therapies and mechanics behind biological processes are invaluable information that aid in systematic analysis of genes (Shalem et al., 2014). In diseases like cancer, which are most of the time caused by growing genetic and epigenetic aberrations, CRISPR/Cas molecular system is

used as a therapeutic tool against the modification of the oncogenic genes (Chen et al., 2019).

Although only one DSB is sufficient to stimulate DNA-repair mechanisms, it is still necessary to introduce more DSBs. In the absence of a repair template, small indels are presented by the NHEJ pathway to the coding sequences that disrupt the protein translation by frameshifting, consequently resulting in the knocking of the gene. HDR can treat DNA repair templates to repair DSB, prominent to the knock-in or insertion of exogenous DNA. On the contrary, CRISPR repression and activation (CRISPRa/i) is based on the dead or inactive Cas endonucleases that target the specific site within the promoter region and target transcriptional repressor complexes (Thomas et al., 2019). In recent times, CRISPR/Cas9 systems are expanded to various areas like farming, agriculture, targeted therapies, and disease patterns. This part reflects the therapeutic aspects of genetic disorders with a significant focus on monogenetic conditions. The ex-vivo methodology includes the removal of a targeted cell populace from the patient's frame. The specific endonuclease is recruited to make desired modifications and then return the genetically modified cell into the patient's body by grafting. This genetically modified grafting method has minimized the issues like immune response and transplant rejection.

Table 3
CoVid-19 Treatment guidelines.

Clinical stage of COVID-19	Chinese recommendations (Jin et al., 2020)	European (recommendations Interim clinical guidance, 2020)	Italian recommendations (Nicastri et al., 2020)
Patients with mild symptoms (fever > 37.5 °C, exhaustion, and no dyspnea) who are suspected or verified	It is necessary to isolate a suspicious patient. Confirmed cases, on the other hand, can be confined or treated in the same room. If the temperature is above 38.5 °C, ibuprofen is advised. Traditional Chinese medicine can help with symptom relief.	It is suggested that you isolate yourself. Paracetamol is indicated for fever. Antivirals should not be used in suspected COVID-19 patients.	Isolation is recommended. No antivirals. Only symptomatic treatment is recommended.
Patients with mild/moderate symptoms who have been confirmed (fever with a persistent cough, no requirement for O ₂)	Ibuprofen is prescribed for fever. Traditional Chinese medicine can help with symptom relief.	As before, symptomatic treatment. Hydroxychloroquine is used as a treatment.	Treatment is symptomatic with appropriate hydration. Treatment options include lopinavir/ritonavir, hydroxychloroquine, and chloroquine.
Confirmed severe instances with elevated respiratory rates and pneumonia.	In the ICU, there is a lot of support. Ibuprofen is prescribed for fever. Traditional Chinese medicine can help with symptom relief. Antiviral drugs are used.	In the ICU, supportive care is provided, as well as the administration of appropriate medications to avoid opportunistic infections. Use hydroxychloroquine chloroquine or lopinavir/ritonavir if hydroxychloroquine is not available.	In addition, ICU care, oral hydration, appropriate antibiotics to prevent opportunistic infection, and adequate peripheral oxygenation are necessary. Start with lopinavir/ritonavir + hydroxychloroquine or chloroquine + tocilizumab* if Remdesivir** isn't accessible.
COVID-19 cases of critical importance (acute respiratory distress syndrome)	Mechanical ventilation, both non-invasive and invasive. Extracorporeal life support is given if the patient does not respond. Vasoactive medicines, empirical antibiotic therapy, corticosteroids (not overused), and antivirals are all utilised to enhance circulation. In the event of septic shock, crystalloids will be administered by IV.	ICU support, mechanical breathing, and broad-spectrum antibiotics. If Remdesivir** is not available, start with HCQ + tocilizumab + steroids.	Mechanical ventilation is used to keep the patient alive, and broad-spectrum antibiotics are used to prevent opportunistic infections. Systemic steroids (methylprednisolone/dexamethasone) are commonly used. If refractory hypoxemia arises, ECMO may be used. If Remdesivir** is not available, start with lopinavir/ritonavir + HCQ or CQ + tocilizumab*.

On the other hand, in-vivo gene rehabilitation includes the direct introduction of genomic transforming factors, like the nucleases of order and patterns in the patient's body (Rezaei et al., 2019). In Cas9 systems, this direct manipulation of endogenous genes resulted in the production of faster and efficient transgenic models that were previously problematic. The transgenic model can be obtained by injecting the Cas9 and transcribing a single RNA into the fertilized zygote to alter the targeted gene sequence to accomplish the desired result in mammalian cells. The Cas9 systems are also utilized for the molecular study, diagnosis and treatments of polygenetic diseases like autism and diabetes due to the multiplexing properties (Wang et al., 2018).

2.2. CRISPR/Cas9 gene-editing technology application against diseases like Covid-19

CRISPR/Cas9 technology is currently more widely implemented in many fields, including food / plant development, drug development, genome technology, biofuel production and genetic engineering technology to fight against new diseases such as Coronavirus disease 2019 (COVID-19) (de Wilde et al., 2018; Ogaugwu et al., 2019; Chen et al., 2019; Shalem et al., 2014; Ai et al., 2019; Strich and Chertow, 2019). The mesovirus, Arterivirus, ronivirus and coronavirus that belong to the family Nidovirales have major effects on society and the economy. The outbreak of highly pathogenic, severe acute respiratory syndrome coronavirus (SARS-CoV) and the Middle East respiratory syndrome (MERS-CoV), in 2002–2003 occurred in zoonotic with high mortality rate and transmitted to the human population rapidly. It has been observed after the outbreak of SARS-CoV, more than 8000 cases were reported in few months with a Mortality rate of 10% (Hui and Zumla, 2019). After the outbreak of MERS-CoV, 2000 cases were reported with a 35% mortality level (<http://www.who.int/emergencies/mers-cov/en/>) (Azhar et al., 2019). And recently at the end of 2019, a pandemic outbreak has arisen in the Wuhan city of China, causing a severe mortality ratio of 35% ([https://](https://www.worldometers.info/coronavirus/)

www.worldometers.info/coronavirus/) (Organization 2020, Huang et al., 2020). In most of the COVID-19 recovered patients, it has been observed that excessive immune response leads to long-lasting lung-damage, fibrosis and functional disability that short the life span. The Covid 19 contamination is related with the cytokine storm (Hui and Zumla, 2019; Azhar et al., 2019; Huang et al., 2020; Zumla et al., 2020; Li et al., 2020a; Channappanavar and Perlman, 2017). Still, there is no effective antiviral drug available in the market against COVID-19 infection. However, it will require many years to develop an effective antiviral vaccine against COVID-19, including the existing antiviral therapies repurposing (Zumla et al., 2016; Beigel et al., 2019; Zumla et al., 2015). Urgent scientific investment and funding, at an advanced level, are required for the interventions of novel therapeutics for COVID-19. The need is to develop proper strategies against the control of nidovirus-induced diseases and increase our understanding of its replication and interaction with host cells. CRISPR-Cas gene-editing technology is the ultimate solution to control these complex RNA viruses. The genome of Nidoviruses span over 13–16 kb (arteriviruses) and 26–34 kb (Coronaviruses) (Gorbalenya et al., 2006; Nga et al., 2011). With composite machinery for the translation of non-structural proteins (NSPs). It also imparts a settled arrangement of subgenomic (sg) mRNAs to express the fundamental underlying proteins (de Wit et al., 2016; Snijder et al., 2013). The viral RNA synthesis is driven by replication and translation complexes (RTCs) that are assembled from NSPs, in complex with the host factors (Gosert et al., 2002; Hagemeijer et al., 2012; Pedersen et al., 1999; van Hemert et al., 2008). It is believed that these RTCs are presumably interconnected with endoplasmic reticulum-derived membrane layer (ERDM) and double-membrane vesicles (Gosert et al., 2002; Knoops et al., 2012; Maier et al., 2013; Ulasli et al., 2010). The repetition of Nidovirus is dependent on transport across the membrane, host cell factors and cycles or host signaling pathway (de Wilde et al., 2017a; van der Hoeven et al., 2016; Zhong et al., 2012). The cyclophilin (Cyp) family that belongs to the Peptidyl-propyl isomerase (PPIases)

superfamily of proteins are known to be involved in nidovirus replication. The Cyps behave as chaperones to encourage protein folding, immune cell activation and transport (Naoumov, 2014, Nigro et al., 2013). The ubiquitous Cyps family that are plentiful cytosolic proteins, particularly CypA, a significant factor involved in RNA viruses replication. Additionally, CypA has a role in immunodeficiency virus 1 (HIV-1) diseases and human hepatitis C infections (HCV). It helps in the processing of HCV polyprotein, remodel cellular membranes into HCV replication organelles by interacting with HCV-NS5A and balances out HIV-1 capsids to advance the nuclear import of the HIV-1 genome (Hopkins and Gallay 2015). Insights into the Cyp inhibitors like cyclosporine A (CsA) demonstrated that cyclophilins were at first embroiled as host features in the reproduction of nidovirus. Cell culture study revealed that coronaviruses and arteriviruses are frequently inhibited by non-immunosuppressive analog NIM-811 and Alisporivie (ALV) and the lower concentration of CsA (Carbajo-Lozoya et al., 2014; de Wilde et al., 2017b; de Wilde et al., 2013a,b; de Wilde et al., 2011; Kim and Lee, 2014; Tanaka et al., 2012; von Brunn et al., 2015). CypA knockdown contribute to the replication of nidoviruses like human coronavirus (HCoV)-NL63 (Carbajo-Lozoya et al., 2014). Feline coronavirus (Tanaka et al., 2017) [64], HCoV-229E (von Brunn et al., 2015). And the arterivirus equine arteritis virus (EAV) (de Wilde et al., 2013a,b). CypA and cosedimented EAV RTCs are directly associated with the RNA synthesis machinery of Arterivirus (de Wilde et al., 2013a,b). Utilized the CRISPR/Cas9 system to investigate three nidoviruses in the same cell line i.e., Huh7 and concluded that their replication process is dependent on CypA knockout. Previously it was also concluded that the replication of Alphacoronavirus HCoV-229E (von Brunn et al., 2015). And Arterivirus EAV (de Wilde et al., 2013a,b). Are dependent over CypA knockdown. However, unlike arteritis viruses, (de Wilde et al., 2018). Reported MERS-CoV's modest replication dependency over CypA using Huh7-CypAKO cell lines. They have reported significant differences in the replication dependency of EAV and other coronaviruses over CypA in the Huh7-cypAKO cell lines. This diversity requires further investigation of the replication dependency role of CypA in other coronaviruses.

2.2.1. The capacity of CRISPR diagnostics

The CRISPR technology plays a vital role in diagnostic capacity with special effectiveness in clinical utility. Many genetic diseases

are diagnosed within animal models around the globe using the CRISPR technique. This has also been extended to treat human inherited disorders, including ex vivo editing, eradicating disease cells and introducing the corrected ones. This ex vivo alteration approach is the most feasible and technically correct approach to treat blood disorders like β -thalassemia, erythrocyte malady and other devastating blood diseases. This strategy also aids in cancer immunotherapies (Foss et al., 2019). The application of CRISPR/Cas systems is not limited to any particular illness, instead spread out to areas like biodefence, fetal medicine and synthetic food. In the past three years, several research articles have pointed out CRISPR's role in diagnosing early-stage cancer and detecting infectious diseases; however, the most prominent news coming out was its role in detecting Tuber Culosis (TB) using CRISPR-based assay (Ai et al., 2019).

2.2.2. Crispr ethics

Moral assessments in biomedicine are majorly driven by the potential risks, benefits, and attempts to reduce risk by maximizing benefits. The formulation, manipulation and updation of ethical principles are made with keeping in view the possible consequences of each decision. Since the development of CRISPR genome editing technology, ethical concerns have been raised on its on-target editing efficiency (Brokowski and Adli, 2019).

Behavioral decisions, especially in biomedicine, are extremely informative and entail examining potential stages of advantage in an effort to maximise profits while avoiding risk. It's vital to think about the extent of prospective outcomes, the practicality of each application, and the possibilities for evaluation outcomes when navigating the decision-making process. After three more imperative enlightenment, ethical concerns about CRISPR technology engineering are addressed with a big release. This includes the possibility of working with detailed planning (Brokowski and Adli, 2019). CRISPR/Cas9 is a low-cost, quick, and precise means to classify genes down to individual nucleotides, as well as a way to test or explain a wide range of scientific concerns. Furthermore, this genetic engineering technology offers new forthcoming technology to cure a number of human ailments, including the novel coronavirus (covid-19).

Furthermore, the application of CRISPR/Cas9 genetic engineering technology and stem cells (i.e., convinced stem cell pluripotent) may aid in the production of gametes for reproductive purposes or

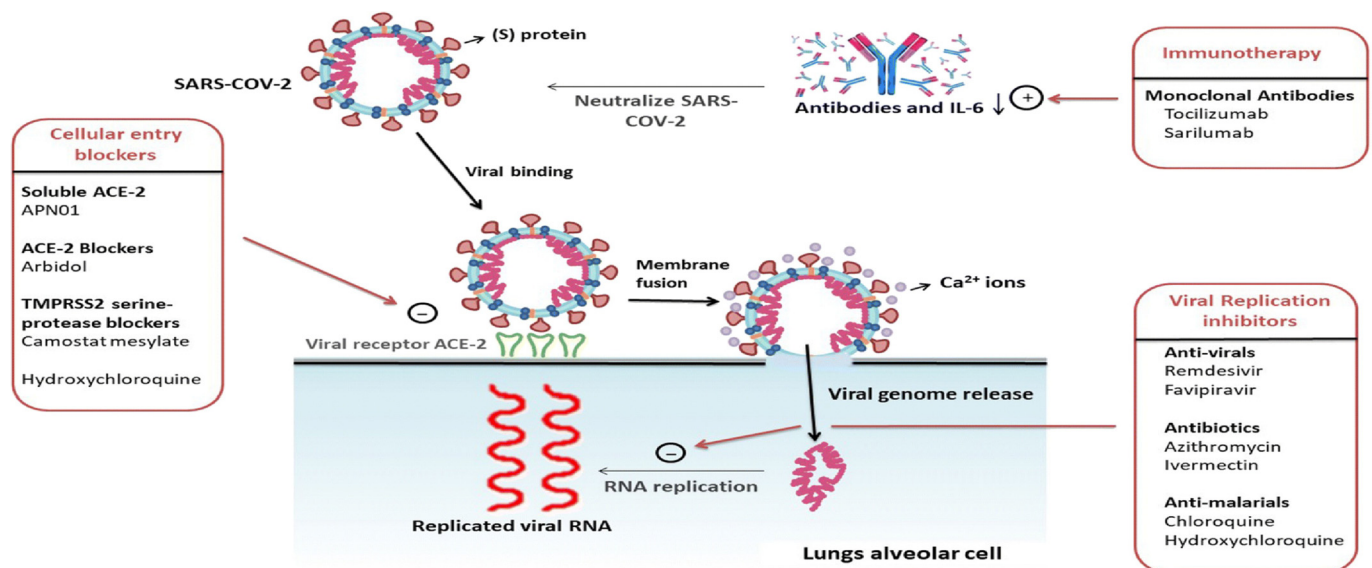


Fig. 2. Mechanism of action of the main repurposing agents against the COVID-19.

to correct flaws in their genome, thus lowering the need for oocyte donation (Sugarmann, 2015). The Committee on Human Gene Editing of the US National Academies of Sciences, Engineering, and Medicine issued updates in 2017 on scientific, legal, and ethical concerns concerning the remarkable benefits of genetic engineering technology. A surprising report, however, was that genetic planning helped to improve gene mutations with the aim of creating a new gene that could pass on genomic mutations to future generations, now not allowed but could eventually be forgiven for certain medical indications. Currently, creating, destroying, or transforming human embryos into genetic mutations for research purposes, is illegal in U.S. government funding. NASEM nonetheless states that once safety risks have been confirmed, plausible clinical trials can begin (Brokowski, 2018).

3. Treatment approaches

3.1. Pathogen-targeting approaches of COVID-19

Even with the fast growth of the COVID-19 epidemic, inopportunistically, antivirals otherwise additional drugs silent are until now toward remaining accepted intended for the COVID-19 treatment. Though the abrupt epidemic has required repurposing the previously nearby medications (designated in Fig. 2), they consume remained standards of preserving action in contradiction of HIV, MERS, and SARS-CoV-1.

3.2. Anti-virals approach of COVID-19

The usage of an existing anti-virus can be a lifesaver when it comes to reducing viral load and treating infections. Many surviving antiviral mediators have attempted to test their anti-COVID-19 activity in vitro (Xu et al., 2020). Protease inhibitors with properties play an important role in the prevention of RNA viruses. Then, in China, a open-label, randomized control trial (ChiCTR2000029539) was created based on the in vitro inhibitory reported effects of lopinavir-ritonavir in contradictions of acute respiratory disease caused by SARS and MERS (Registry CCT, 2020a, Rasheed et al., 2021).

In vitro antibodies against COVID-19 have also been found in nucleoside physical analogues such as favipiravir, remdesivir, and rabavirin. A recent study looked into the role of five anti-viruses in limiting COVID-19, a virus that suppresses viral amalgamation and reproduction in Vero E6 cells. Remdesivir, which has a low half-maximum effective concentration (EC50) of 0.77 μ M, has been discovered to have significant antiviral activity, as has the anti-malarial mediator chloroquine, which has an EC50 of 1.13 μ M (Wang et al., 2020a,b). Remdesivir was developed with the help of Gilead Sciences, an American-based company, and has previously been used to eradicate the Ebola virus (Mulangu et al., 2019). Because of the aforementioned success in vitro Vero E6 cell checking out, it's a multidisciplinary antiviral investigative mediator that's currently outperforming placebo-controlled clinical studies and even experimental testing in phase iii. Overall, it is currently being used in COVID-19 patients in a variety of settings in the United States and overseas (NCT04292899) (NIH, 2020r). As a result of COVID-19, Gilead Sciences and Pakistan's national pharmaceutical industry, ferozsons laboratories Ltd., have agreed to a deal that will allow Pakistan's national pharmaceutical industry, ferozsons laboratories Ltd., to produce a product for domestic use as well as distribute and export to other countries (Reuters, 2020). Provisionally, the only antimicrobial drug that can be recommended for patients with low oxygen ranges (less than 94%) and patients with extreme respiratory infections. However, its use in patients with minor ailments is not recommended. In recent

cases of hospitalized patients clearly showed that the mortality rate was significantly reduced (from 11 to 7.1%) and remdesivir reuse (Beigel et al., 2020). According to study literature, arbidol, a tiny monocular mediator with antiviral action against influenza virus strains, adenovirus, rhinovirus, and a variety of other DNA and RNA viruses, also appears to have anti-inflammatory effect. COVID-19 also evaluates current Phase IV clinical trials (NCT04260594) (NIH, 2020e).

3.3. Anti-malarials drugs for COVID-19

Based on its low EC50, 1.13 μ M, chloroquine, a long-used anti-malarial and anti-rheumatic drug, has been discovered to have strong anti-COVID-19 action (Wang et al., 2020a,b). The fact that chloroquine had good in vitro results encouraged scientists to give it to the human subjects in his studies. Since his low EC50 also advanced cytotoxic medication CC50, chloroquine has been widely used in human clinical trials at more than ten Chinese hospitals. The outcomes in terms of viral weight loss, illness duration, and COVID-19 pneumonia exacerbation have yet to be determined (Gao et al., 2020). Chloroquine's capacity to prevent the virus from joining and entering the patient cell is the only probable mechanism for its anti-COVID-19 properties.

(Rabi et al., 2020). Though, because of the chloroquine's toxicity-mediated limits, its takes not been presented in the scientific trials through the NIH. The Hydroxychloroquine, an appropriate another exposed to significantly not as much of toxic (app. 40%) also consuming similar of its anti-COVID-19 activity on his parental medication, the presently experiencing randomized skillful stage III of this scientific trials (NCT04261517) (Yao et al., 2020, NIH 2020f). The fundamental of the antiviral mechanisms of Hydroxychloroquine remains a rise in the endosomal pH also his inhibition of virus entrance interested in the target cell (Savarino et al., 2003). Inappropriately, not any substantial indication remained throughout the current trials to provide the curative usage of the chloroquine and Hydroxychloroquine for COVID-19 (Sanders et al., 2020).

3.4. Antibiotics approach used for COVID-19

A randomized CONTROLLED OPEN LABEL study was done in China, evaluating the burden of post-treatment viral load on three randomized groups treated with hydroxychloroquine and azithromycin, hydroxychloroquine alone, and ordinary care. The study's findings clearly demonstrated the efficacy of combination therapy (100%) over hydroxychloroquine monotherapy (57.1%) and general care (2020-000890-25) (Register ECT, 2020). The combination use of hydroxychloroquine and azithromycin in Covid-19 patients is related with a considerable reduction in viral capacity and fatality rates, according to the findings of a recent non-randomized controlled experiment in Michigan, USA. The possessions, on the other hand, are significantly less effective and concrete due to the research study's applied limitations and hypothetical current NIH sanctions, which advocate avoiding their usage in COVID-19 (Arshad et al., 2020). According to recent sources, another macrolide, clarithromycin, is undertaking a phase IV experimental test trial at Beijing YouAn Hospital, led by Ronghua Jin (NCT04286503) (NIH, 2020s).

3.5. Anti-parasitics drugs used for COVID-19

The Nitazoxanide remains an agent previously demonstrated by the approach of an antiparasitic medicine that likewise has a strong COVID-19 inhibitory effect, but with qualities less favourable to Remdesivir patients (Wang et al., 2020a,b). Newly, Australian scientists have established the noticeable in vitro

antiSARS-CoV-2 activity of the ivermectin. The remains similarly have active in contradiction of the human immunodeficiency virus (HIV) dengue virus. The antiparasitic representative consumes remained to originate toward significantly decrease the viral RNA stages, the identified using of RT-PCR, on the attention is about two μ M, maybe due to the reserve of the IMP α / β 1, the viral protein transporter complicated of the replication (Caly et al., 2020, Wagstaff et al., 2012).

3.6. Anti-COVID-19 small molecule medications—blocking cellular entrance

Because of the rising range of COVID-19 contamination around the world, now is the moment to progress the unique anti-COVID-19 mediators. Camostat mesylate, a potent protease inhibitor previously approved in Japan for a variety of conditions including pancreatitis and influenza, has demonstrated antiviral activity by preventing viral entry into the host cell by lysis of TMPRSS2, a serine protease linked to the COVID-19 spike (S) protein (Hoffmann et al., 2020). Inappropriately, there is still a scarcity of data, studies, and trials that have advanced drugs in violation of COVID-19. Arbidol (umifenovir), a different chemical, is nonetheless thought to impede COVID-19 endocytosis in the host cell. This is why, despite the COVID-19, Arbidol is still being studied in several research trials (Liu et al., 2020). The Chinese scientific trial of activities laterally by the NIH is leading trials on the efficacy of Arbidol, and is also alone otherwise by the protease inhibitor favipiravir (NCT04260594, ChiCTR2000030254) (Chen et al., 2020). The needed of the SARS-CoV-2 spike protein is resolved by ACE2 par-enteral management, which is shadowed by the reserve of cellular endocytosis (Kuba et al., 2005). This push would result in a significant improvement in the lung cellular wound. These encouraging findings of human recombinant ACE2 (APN01) were assessed to be crucial to a projected decrease in AN-II-mediated lung damage and IL-6 stages. That idea was still plenty to take care of a RhACE2 scientific experiment. Peiron Biologics is currently sponsoring scientific research on the efficacy and also care of the APN01, which is still in stage II (NCT04335136).

3.7. Immunity transformers—a host-targeting approach

3.7.1. Natural killer cells

He consumes remained theorized that there remains a high death rate among senior people due to their insufficient immune response. Therefore, just steadying the patient's immune profile might support a previous resolve of COVID-19 signs. CD4 + T cells,

CD8 + T cells, and antibodies were requested to be preserved in order to reduce SARS-COV-related symptoms (Chen et al., 2010). Therefore, natural killer cells can remain the sensible applicant for the scientific trials presently in stage I (also care efficacy calculation) at current the (NCT04280224) (NIH, 2020k).

3.8. Suggestive treatment approaches

3.8.1. Slight infection approach

Symptomatic patients with mild COVID-19 (fever > 37.5 °C, fatigue, but no breathing difficulties) should isolate suspected confirmed cases in the quarantine area. In addition, symptomatic relief is recommended. Fever is typically treated with paracetamol and/or nonsteroidal anti-inflammatory drugs, and there is no specific treatment at this time. Furthermore, Chinese doctors and health professionals advise patients to use Chinese medicine to treat their illnesses (Yang et al., 2020). European guidelines, on the other hand, have confirmed the use of hydroxychloroquine (HCQ) in COVID-19 patients. More information can be found in Table 4. (Nicastri et al., 2020).

3.9. COVID-19 pneumonia approach

Increased breathing rate, lung ventilation, and active symptomatic therapy should be explored for individuals with cough and fever. In this case, broad-spectrum antibiotics should be considered to prevent opportunistic lung infections and proper oral hydration use. In order to reduce pulmonary secretions, it is recommended that the Chinese study also use selective M3 blockers. Furthermore, if antimalarials are contraindicated, anti-COVID-19 norms treatment with lopinavir or ritonavir is recommended at this time (Jin et al., 2020, Nicastri et al.2020).

3.10. Recent advances in the symptomatic approach

3.10.1. Treatment—antithrombotic therapy

After a more in-depth investigation of COVID-19 medicine, several specialists detected the emergence of dispersed intravascular coagulation (DIC) and venous thromboembolism (VTE) in COVID-19 patients through severe pulmonary participation evidenced by raised D-dimer levels (Lauer et al., 2020). Additionally, pulmonary thrombosis might occur as a result of this coagulopathy (Marongiu et al., 2020). Several therapeutic techniques, particularly traditional DIC treatment options, have been tested to control COVID-19-induced coagulopathy. Tissue plasminogen activators (TPA) were used to define a scenario sequence, however among the three

Table 4

Nature of vaccine	Target of vaccine	Principal developer of the vaccine	Country	Clinical trial status	Reference
DNA Vaccine (INO-4800)	Spike (S) Protein	Inovio Pharmaceuticals	USA	Phase I NCT04336410	(NIH, 2020q)
non-replicating virus	Spike (S) Protein	University of Oxford	UK	Phase I/II trial NCT04324606	(NIH, 2020b)
Inactivated vaccine	Entire virus	Shenzhen Geno-Immune Medical Institute	China	Phase I/II NCT04276896	(NIH, 2020j)
		Sinovac Research and Development Co. Ltd.	China and Brazil	Phase II NCT04352608	(NIH, 2020p)
		Wuhan Institute of Biological Sciences	China	Phase I ChiCTR2000031809	(Registry CCT, 2020g)
mRNA vaccine	Spike (S) Protein	Moderna, USA	USA	Phase I NCT04283461	(NIH, 2020n)
Recombinant vaccine (adenovirus type-5 vector)	Spike (S) Protein	CanSino Biologics	China	Phase I clinical trial Completed NCT04313127	(NIH, 2020l, Zhu et al., 2020)
Attenuated live vaccine	Entire virus Serum	Institute of India in collaboration with Codagenix	India and USA	Pre-clinical/animal studies	(AReNA CT, 2020)

focuses, one patient showed a significant increase in ARDS (Wang et al., 2020a,b). Additional DIC therapy options may work in a similar way to limit the progression of secondary disorders, the most serious of which may be heart dysfunction. Aspirin's known cardioprotective qualities were observed in an NIH research trial (NCT04365309) conducted at Xijing Hospital in China (NIH, 2020m). In stage III clinical trials, enoxaparin, for example, continues to show good anti-coagulation in COVID-19 patients with DIC and otherwise VTE Barnes (Barnes et al., 2020).

3.11. Convalescent plasma/immunoglobulin utilisation approach

Better patients' plasma contains immunoglobulins that can be used to treat individuals with dynamic viral disease. In 2014, the National Institutes of Health (NIH) continued to fund scientific experiments to calculate recovering plasma treatment in the face of MERS-COV-induced ARDS. The scientific trial was discontinued at stage II (NCT02190799) despite the fact that the results were still inadequate (NIH, 2020c). This method of treatment has also been tested in the case of the Ebola virus in Africa. Furthermore, based on knowledge of immunoglobulin responses gained over the last two years from trials aimed at developing viral respiratory illnesses. The FDA uses recently accepted scientific tests to assess the efficacy and safety of this treatment technique in the current ailment. Recently, a randomised scientific trial was approved to evaluate the benefits of convalescent plasma in COVID-19 individuals who were censoriously ill; also, they looked with repercussions to show slightly otherwise no development. Furthermore, the disease time does not appear to be summarised by adding COVID-19 to the normal treatment (Li et al., 2020b). However, there are still hazards connected with immunoglobulin treatment, which is why, according to the FDA, it must be given specifically for severe, otherwise life-threatening COVID-19 (Administration USFD, 2020).

3.12. Immunization: Channel vaccines approaches

This virus is particularly contagious due to its high level of person-to-person transmission. Preventive defence trials and the use of a vaccination are the two most effective habits for reducing the spread of an epidemic. After our review's main worry, vaccination remains the backbone for the inhibition of slightly viral epidemic disease. Maximum basically, the vaccine must be essential to remove the high spreadability of COVID-19, which uncertainty not measured will remain to determine the present epidemic. The spike (S) protein linked to COVID-19's membrane is the most commonly chosen target for advancing a COVID-19 vaccine (Liu et al., 2020). In defiance of COVID-19, a variety of stages are currently used in vaccine research. Table 4 lists key COVID-19 pipeline vaccine developments around the world. NIH currently lists six immunisation trials, with the majority of them taking place in the United States, the United Kingdom, and China.

4. Conclusions

Based on the current assessment and evaluating literature inquiry, we can accelerate the most critical factors of CRISPR RNAs (crRNAs) and CASE protein effector. CRISPR will be a highly fundamental, decisive, and vital research point for disease diagnosis and treatment in the future, with enormous potential for scientific research. Apart from that, it will be crucial in preventing gene and genetic mutations, as well as transmissible diseases; it will also be necessary for viral tolerance to repair immune cells that fail to recognise, resulting in immune system failure. Prompt identification and quick care of this lethal disease are critical and required measures when using COVID-19 epidemic. The current primary

diagnostic procedure has various drawbacks, including lack of durability and lengthy processes. Substitute nucleic acid recognition capabilities, such as reverse transcriptase-mediated isothermal amplification (RT-LAMP) and CRISPR, as well as immunoassay-based devices, are presently in the patenting stage. In accumulation, there is an imperative need for drug improvement to switch this lethal, worldwide epidemic, which is precarious for the whole international community. The high mortality rate and spread of COVID-19 necessitate the use of appropriate medications and vaccines as critical tools in preventing the disease's spread. Several organizations with approved indicators are currently being tested for COVID-19 treatment. Despite the fact that there has been insufficient progress in this area, treatment with remdesivir and dexamethasone has resulted in a promising response to caution. In addition, clinical trials are still being carried out on a number of medications. In addition, six vaccines are now being tested in clinics on an experimental basis, but recommending and selling safe and effective vaccines will take a year or more.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

The authors extend their appreciation to the Deanship of Scientific Research at King Khalid University, Abha 61413, Saudi Arabia for funding this work through research groups program under grant number R.G.P-1-223-42.

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